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(71) Applicant: HYBRITECH INCORPORATED [US/US]; 11085 Torreyana Road, San Diego, CA 92121 (US).

(72) Inventors: MARTINIS, Joanne ; 2095 Redbird Drive, San Diego, CA 92123 (US). BARTHOLOMEW, Richard, M. ; 13143 Sundance, San Diego, CA 92129 (US). DAVID, Gary, S. ; 9477 Poole Street, La Jolla, CA 92307 (US). ADAMS, Thomas, H. ; 860 Neptune, Leucadia, CA 92024 (US). FRINCKE, James, M. ; 339 Shoemaker Lane, Solana Beach, CA 92705 (US).

(74) Agents: RESPESS, William, L. et al.; Lyon & Lyon, 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US).

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(54) Title: ANTIBODIES HAVING DUAL SPECIFICITIES, THEIR PREPARATION AND USES THEREFOR

(57) Abstract

Described herein are 'polydomas', the product of the fusion of a hybridoma with a B-lymphocyte or another hybridoma. The polydomas produce a hybrid monoclonal antibody having a dual specificity against two different antigenic determinants. Also described are immunodiagnostic and immunotherapeutic processes which utilize the hybrid monoclonal antibodies or other antibodies having a dual specificity. In those processes, one specificity of the antibody is directed against a target antigen and the other against a moiety which permits a diagnosis to be made or which delivers an agent lethal to the target antigen or associated tissue.

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DESCRIPTION

ANTIBODIES HAVING DUAL SPECIFICITIES, THEIR PREPARATION AND USES THEREFOR

Field of the Invention

This invention relates to antibodies having dual specificities. In another aspect it relates to immunodiagnostic and immunotherapeutic processes. In yet another aspect it relates to hybridomas and related monoclonal antibody technology.

Background of the Invention

The antigen-antibody reaction is already routinely exploited in a variety of practical applications and is being widely investigated to establish its value in other, as yet unproven, utilities. For example, serum antibodies produced by a host animal's immune response to an immunogen can be used in affinity purification procedures to isolate the immunogen from solutions in which it is present in only minute quantities.

In other circumstances, if the immunogen is a disease associated antigen, its presence in a patient's serum or other body fluid can be detected using immunoassay or immunometric techniques. For example, detection of HBsAg using a radioimmunometric technique is the current method of choice. On another front, serum antibodies to ferritin, obtained from New Zealand white rabbits and labeled with ^{131}I, have been reported as showing promise for the treatment of liver tumors. (See Order et al, International Journal of Radiation Oncology, Biology and Physics, 6, 703 (1980)).

Serum antibodies, for example, those obtained from rabbits, murine species or other mammals are "polyclonal" in nature since the immune system of the host is stimulated to produce a mixture of specific antibodies directed

to the different antigenic det rminants or epitopes on the immunogen to which the host is responding. The individual antibodies making up the mixture are each the product of a B-cell clone; furthermore each B-cell secretes only one antibody specie. The antibody produced by one clone differs from an antibody against the same antigen produced by another clone by having at least a subtle difference between its peptide sequence and that of the other antibody. In effect, therefore, each antibody specie is a distinct molecule and the differences in peptide sequence between different species affect their general specificities as well as the particular epitopes they recognize and their affinities for the antigen.

An individual B cell cannot be grown indefinitely using presently available tissue culture techniques to obtain the antibody specie it secretes as a pure compound. Relatively recently, Kohler and Milstein discovered and reported a process by which a monoclonal antibody can be conveniently obtained as the secretion product of a hybrid cell referred to as a "hybridoma". (G. Kohler and C. Milstein, Nature, 256, 495 (1975)). Basically, the process involves the fusion of spleen cells taken from an immunized mouse with mouse myeloma cells to form the hybridoma. Myeloma cells which do not produce, or at least do not secrete their own immunoglobulin or parts thereof are preferred. Cultures of cells obtained by cloning a single hybridoma will secrete identical antibody molecules which can subsequently be obtained readily as a pure chemical compound. This is in contrast to the conventional antibody preparation obtained, for example, from serum, in which any one antibody is but one of the components of a substantially unresolvable antibody mixture of related, yet distinct chemical compounds.

Since it is a pure compound, a monoclonal antibody will have a constant specificity for a single site on the antigen molecules and a well defined affinity. Thus,

clones of different hybridomas can be screened to select the one producing the monoclonal antibody with the most desir able properties for a given application. The immortality of the hybridoma guarantees an almost unlimited supply of the antibody it secretes and all viates problems associated with variance in antibody titer and overall affinity from animal to animal used to produce serum antibodies. Monoclonal antibodies obtained from hybridomas have, for example, been put to practical application in diagnostic kits. A selection of such kits is available from Hybritech, Inc., assignee of this application.

An antibody molecule can generally be considered to express a single specificity which is exhibited towards the immunogen to which the host's immune system responded by production of the antibody. The antibody is composed of two identical halves, each of which is comprised of a heavy and light chain pair and each of which recognizes the same antigenic determinant as the other. The following is a representation of the arrangement of heavy (H) and light (L) chains in an antibody molecule:

The -S-S- disulfide bridges which link the two (H) chains together at the location of cysteine moieties can usually be cleaved selectively <u>in vitro</u> by a mild reduction, and the half molecules disassociated by subsequent acidification. The half molecules can th n be recombined

(renatured), again in vitro, at neutral pH, the reassociation taking place through non-covalent interaction.

If antibodies of different specificities are subjected to a selective cleavage of the disulfide bridges between the heavy chains and conditions conducive to renaturation subsequently established, reassociation between half molecules may occur randomly to produce a population of antibodies, at least some of which are hybrids in that one half of an antibody molecule of one specificity combines with one half of an antibody molecule of a different specificity. For example, in Nisonoff et al, "The Antibody Molecule", Academic Press, New York (1975), at pages 260-261, is described an in vitro production of a polyclonal antibody hybrid of rabbit anti-ovalbumin and anti-BGG antibodies. Hybrid monoclonal antibodies have also been obtained using an analogous process. See D.M. Kranz et al, Proc. Natl. Acad. Sci. USA, 78, 5807 (1981). Theoretically at least, the hybrid antibody will exhibit a dual specificity in that one half of the antibody will recognize and bind to one antigenic determinant or epitope, whereas the other half will recognize a different epitope on the same or a different antigen.

Although hybrid antibodies can be obtained in the manner described above, the yields are often very low, the reactions used to make them difficult to reproduce and the hybrid antibodies usually suffer significant, irreversible denaturation. Such denaturation can reduce immunoreactivity and would be expected to result in different metabolic characteristics in vivo. As a result, the hybrid antibody today remains largely a laboratory curiosity which is difficult to obtain.

Antibodies having dual specificities may also be prepared by conjugating pairs of intact antibodies, monoclonal or otherwise, using a variety of coupling or crosslinking agents such as protein A (from Staphylococcus aureus), carbodiimide and bifunctional compounds such as

N-succinimidyl-3-(2-pyridyldithio) propionate to obtain dimeric and higher antibody multimers to which each member of the antibody pair contributes its specificity. For example, Mandoche et al have reported the formation of multivalent antibodies having dual specificities, by a sequential reaction of antibodies with protein A, which have been shown to be capable of detecting cell surface antigens in vitro. See J. Immunological Methods, 42, 355, (1981). According to their method, antibodies of one specificity bind to the surface antigen and the others to a moiety which permits detection.

The synthesis of dual specificity antibodies by the foregoing techniques is complicated and thus far no commercial application of them has been made.

Summary of the Invention

The present invention provides, among other things, a novel, completely biological method for reliably obtaining hybrid monoclonal antibodies in good yields without denaturation. Throughout this specification, the term "hybrid antibody" will be used to designate a single antibody molecule having two different specificities. The individual specificities may be to antigenic determinants on two different antigens or to different antigenic determinants (epitopes) on the same antigen. Furthermore, unless otherwise indicated, the term "antigen" also embraces haptens.

According to the method of the present invention, hybrid antibodies having a dual specificity are obtained by fusion of a hybridoma, preferably a selectively destructible hybridoma, which secretes an antibody against a preselected antigenic determinant with a fusible B-lymphocyte or a second hybridoma, the B-lymphocyte or second hybridoma secreting a second antibody against a different antigenic determinant, to form a second generation hybridoma (hereinafter "polydoma"). As used herein, the term "selectively destructible hybridoma" means a hybridoma

which lacks, or at least substantially lacks, the capability of surviving in the medium in which the polydoma is cultured. We have unexpectedly found that, unlike the parent hybridoma or the B-lymphocyte from which it is derived, each of which secretes a population of identical antibodies having a single specificity, the polydoma in addition secretes a high percentage of a monoclonal hybrid antibody having a dual specificity, i.e., a capability to bind with either of the antigenic determinants recognized by the individual antibodies produced by the parent cells or with both determinants at the same time. The hybrid monoclonal antibody obtained in this way has not suffered the undesireable denaturation which characterizes hybrids obtained from the process of chemical recombination of antibody half molecules. Furthermore, the process of the invention permits the hybrid to be obtained reliably and in large amounts.

Also according to the present invention there are provided processes for immunodiagnosis and immunotherapy employing antibodies having a dual specificity. Generally these processes employ a monoclonal antibody or polyclonal antibodies having a first specificity against a target antigen and a second specificity against a substance, for example, another antigen or hapten, which permits a diagnosis to be made of the target antigen or which permits delivery of, or is itself, an agent which is lethal to the target antigen or the tissue with which it is associated.

Thus, by an appropriate selection of parent cells, a polydoma can be obtained according to the present invention which will secrete an antibody having one specificity for a target antigen and a second specificity for a moiety useful in diagnosis or therapy. Alternatively, antibody half molecules can be recombined using <u>in vitro</u> chemical means or individual intact mono-specific antibodies can be coupled or crosslinked by chemical means to obtain antibody multimers (which may be a dimer, trimer or higher

multimer) having a dual specificity and having the same or a similar utility as a hybrid monoclonal antibody having the same dual specificity made according to the present invention.

As used herein, the terms "antibody" includes antibody fragments having immunochemical properties such as Fab or $F(ab)_2$ fragments.

Accordingly, an object of the present invention is to obtain hybrid monoclonal antibodies reliably and in good yield that have not been denatured in the process of their preparation.

Another object of the present invention is an improved process for obtaining hybrid monoclonal antibodies.

Yet another object of this invention is to provide immunodiagnostic and immunotherapeutic processes which employ antibodies having a dual specificity.

The manner in which these and other objects can be obtained will be apparent from a consideration of the following description of preferred embodiments.

Description of Preferred Embodiments

As indicated above, the process for obtaining a hybrid monoclonal antibody according to the present invention requires, as one parent, a hybridoma, and preferably a selectively destructible hybridoma, which secretes a monoclonal antibody against a preselected antigenic determinant or epitope. The use of a selective destructible hybridoma as a parent has the advantage that it prevents the cells obtained by fusion of the selectively destructible hybridoma with a B-lymphocyte or a second hybridoma, i.e., the polydoma, from being overgrown by a population of the parent hybridoma when the cells obtained in the fusion process are cultured and to provide a means by which the polydoma cells can be isolated from parental hybridoma c lls.

We have found that selectively d structible hybridomas useful in our invention can be obtained from hybridomas secreting an antibody having one of the desired specificities made by the classic Kohler-Milstein process, i.e., hybridomas obtained by fusion of a myeloma cell and a B-lymphocyte such as that found in the spleen cells of a mouse. According to one embodiment of the invention, such a hybridoma is subjected to a back selection process to obtain the hybridoma which is selectively destructible.

Generally, selective destructibility can be obtained by back selection to a hybridoma which lacks a genetic component which is necessary to its survival in a medium of choice in which the polydoma produced by the fusion can be cultured because of a genetic contribution from the fusion partner of the selectively destructible hybridoma, i.e., the B-lymphocyte or second hybridoma.

The presently preferred back selection process involves culturing a hybridoma which secretes an antibody having one of the desired specificities to be incorporated into the hybrid antibody in a growth medium containing 8-azaguanine. In such a medium, any cell which incorporates 8-azaguanine and can, therefore, grow in the medium are ones which lack the enzyme hypoxanthine-guanine phosphoribosyl transferase (HPRT). Clones of cells which lack this enzyme cannot grow in medium containing hypoxanthine aminopterin thymidine (HAT). Thus, they can now be selectively destroyed in that medium.

A very similar process for back selection involves growing the hybridomas secreting the desired antibody in a medium containing 6-thioguanine, another analog of guanine toxic to the cell if incorporated into the DNA. Again, certain cells which will grow in this medium lack the HPRT enzyme and clones of these cells will necessarily be sensitive to HAT medium.

Yet another process for back selection which can be used in the invention involves growing cells of the selected hybridoma cell line in a medium containing either

of the thymine analogs 5-bromouracyl deoxyribose (BUdR) or 2-aminopurine. Only those cells lacking the enzyme thymidine kinase (TK) can grow in a medium containing either of these two inhibitors. As in the case of cells lacking the enzyme HPRT, cells lacking TK will not grow in HAT medium.

A different process for obtaining a selectively destructible parent hybridoma involves irreversible enzyme inhibition using metabolic inhibitors. Among these, the so called K_{cat} inhibitors are preferred. These inhibitors are analogs of an enzyme's substrate which are converted by the target enzyme into a highly reactive molecule which reacts with the enzyme at its active site resulting in irreversible inhibition of the enzyme. For example, treatment of the selected hybridoma with an analog of glutamine such as azaserine or 5-diazo-5-oxa-L-norleucine (DON) irreversibly inhibits the enzyme formyl-glycinamide ribonucleotide amidotransferase by formation of a covalent bond with a cysteine residue at the enzyme's active site. This inhibition will ultimately result in cell death. However, the hybridoma can be rescued by fusion with the second parent of the polydoma which supplies the necessary enzyme.

In a preferred embodiment, the selectively destructible hybridoma is fused with complementary B-lymphocytes, typically obtained as spleen cells taken from a host which has previously been immunized with an antigen, which may be a hapten bound to a carrier protein, selected to cause the host to generate an immune response which produces antibodies having the second specificity desired in the hybrid antibody. The host is usually a mouse but species of rabbits, humans and other animals may also be used although interspecies fusion may exhibit a low order of stability. The process for immunizing such a host is, of course, well known and details need not be given here.

Fusion of the selectively destructible hybridoma with the B-lymphocyt s to obtain the polydoma can be accomplished by combining the two groups of cells in a medium containing an agent known to promote cell fusion such as polyethylene glycol or Sendi virus according to known methods.

After fusion, the cells are transferred to a medium such as HAT medium for culturing. The B-lymphocytes will survive for only a brief period of time and the parent hybridoma cells cannot grow in the medium. However, the population of polydomas formed as a result of the fusion, because of complementation of the parent hybridoma by the B-lymphocyte, for example, by a genetic contribution of the ability to make a missing enzyme such as HPRT or TK or by a direct contribution of an enzyme inhibited in the parent hybridoma, can be grown in the medium. Clones of individual polydomas are cultured and screened to select those which secrete antibodies having the desired dual specificity. Clones of polydomas whose antibodies exhibit the desired dual specificity are further screened to select those whose second specificity, i.e., that obtained from the B-lymphocytes, and affinity are most desireable.

In another embodiment, the polydoma is obtained by fusing the selectively destructible hybridoma using a suitable fusion agent with a second hybridoma which is also selectively destructible. The second parent hybridoma is obtained in the same manner as the first, i.e., by a process of back selection, irreversible enzyme inhibition or by any other suitable technique. In such a case, the second hybridoma must be able to complement the first. For example, if the first selectively destructible hybridoma lacks the enzyme HPRT, the second must be capable of contributing to the polydoma a gene which will enable the polydoma to express HPRT. Similarly, if the second selectively destructible hybridoma lacks the enzyme

TK, the first must contribute a gene for TK to the polydoma. Similar complementarity between the two hybridomas must be present if irreversible inhibition of an enzyme has been accomplished to confer selective destructibility on them. It is also possible to use, as one hybridoma parent, a hybridoma which has been subjected to a back selection process, and, as the other, a hybridoma which has been subjected to a process of enzyme inhibition.

The use of complementary selectively destructible hybridomas as parents for the polydoma has the advantage that both parents can be selected on the basis of th specificities and affinities of the monoclonal antibody they produce whereas, in the case of fusion of a single hybridoma with B-lymphocytes, no pre-fusion selection among the B-lymphocytes to obtain those producing an antibody of the desired specificity and affinity can be made.

Fusion of the two selectively destructible hybridomas can be accomplished using polyethylene glycol or using other fusing agents, again according to known methods. After fusion, the cells are transferred to a growth medium in which the two parents cannot grow, but in which the polydomas resulting from the fusion are capable of growth because of the complementary contributions of the parents.

Heretofore, we have discussed selection processes to obtain selectively destructible hybridomas for use as both of the hybridoma partners in a hybridoma-hybridoma fusion to form a polydoma. However, the necessity for the second hybridoma parent to be selectively destructible can be avoided by conferring both a dominant and a recessive marker on the first hybridoma parent. A presently preferred method is HAT-ouabain selection. The drug ouabain is a specific inhibitor of the Na^+-K^+ activated ATPase of the plasma membrane. That enzyme is responsible for the importation of K^+ into a cell and the export of Na^+ from the cell. Cells of a hybridoma previously back selected to confer selective destructibility, for example,

HAT sensitivity, are grown in ouabain medium to select for ouabain resistant cells. Clones of these cells will be HAT sensitive but ouabain resistant. By contrast, the hybridoma selected for fusion with it will be ouabain sensitive but can survive and grow in HAT. Alternatively, selection for ouabain resistance can be done first either on the parental myeloma line or the hybridoma derived therefrom, followed by back selection or other technique to confer selective destructibility.

Cells obtained by fusion of the two hybridomas in polyethylene glycol or other fusion agent are transferred to HAT medium containing ouabain in a concentration lethal to the second hybridoma parent. The selectively destructible hybridoma parent cannot survive in the HAT medium either, lacking, for example, the HPRT or other enzyme, even though ouabain resistant. However, the polydoma cells can grow in the medium since they will possess the enzymes and ouabain resistance necessary for survival. The foregoing method has the advantage that it is possible to obtain a polydoma by directly fusing a selectively destructible hybridoma parent secreting an antibody having one of the specificities desired in the hybrid with a second, "off the shelf" hybridoma secreting an antibody having the other specificity desired in the hybrid and no use of techniques for conferring selective destructibility on the second hybridoma parent is necessary.

Yet another technique for obtaining a polydoma which employs a universal parent, i.e., one which has both a positive and a negative marker, which can be fused with any "off the shelf" hybridoma, involves the use of recombinant DNA vectors carrying various drug resistance markers. For example, SV40 carrying a gene for neomycin resistance can be used.

A presently preferred universal parent is one that is HAT sensitive-neomycin resistant. The chosen parent is back-selected to HAT sensitivity and then transfected with SV40 vector carrying a gene for neomycin resistance. This

procedure can also be reversed with transfection being done first. The resulting hybridoma can grow in the presence of neomycin, which is normally toxic to mammalian cells, but will die in the presence of HAT. Off-the-shelf hybridomas, however, grow in HAT but die in the presence of neomycin. Products of the fusion of the parents, therefore, survive in the presence of HAT and neomycin. While the use of vectors to convey resistance to neomycin is presently preferred, vectors carrying genes which will confer resistance on mammalian cells to other drugs can also be used.

Even though presently preferred, it is not essential to our process for obtaining polydomas from pairs of hybridomas that at least one of the parent cell lines be selectively destructible. It is within the scope of our invention to fuse a pair of hybridomas, neither of which is selectively destructible but which secretes an antibody having one the specificities desired in the hybrid, in the presence of a suitable fusion agent followed by the subcloning of all cells before the population of unfused parent hybridomas increases to an extent that screening the subclones to identify polydomas is not practical. The subclones are subsequently screened to establish which secrete antibodies having a dual specificity.

This process is best suited to obtaining polydomas when the fusion frequency of the parent cell lines is high. In any case, and particularly when the cell fusion frequency is low, the cells obtained from the fusion of hybridomas whose monoclonal antibodies are against different antigens can be screened using a cytofluorograph to identify the polydomas. To accomplish this, samples of the two antigens are tagged with different fluorescing moieties whose fluorescence occurs at different wavelengths. For example, one can be tagged with fluorescein and the other rhodamine. The population of cells from the fusion, which have preferably been cultured overnight

or for any other suitable period to increase their numbers, are incubated with the two tagged antigens. The cells are then screened using the cytofluorograph. Those cells which fluoresce at only one of the two wavelengths will be from the cell lines of the parent hybridomas. However, cells which exhibit fluorescence at both wavelengths will be polydomas which can be isolated and subcloned.

In yet another embodiment, a polydoma can be obtained directly by removing the nucleus from a first hybridoma which secretes a monoclonal antibody having one of the specificities desired in the hybrid and inserting it into the cytoplasm of a second hybridoma which secretes a monoclonal antibody having the second desired specificity. Of course, neither of the parent hybridomas needs to be selectively destructible in order to be used in this process. After insertion of the nuclear material, the cell is cloned to obtain a population of the polydoma.

We have found that, unlike the parent hybridomas or B-lymphocytes which secrete a single antibody, polydoma cells obtained according to our invention secrete a mixture of antibodies, at least one of which is a hybrid antibody having a dual specificity. Also produced by the polydoma are relatively smaller amounts of antibodies of the same specificity as those produced by the parent cells used to obtain the polydoma. The ratio of hybrid to mono-specific antibodies appears to be about 2:1:1 which is that expected if the polydoma produces equal amounts of all the possible (H) chains synthesized by the parent cells which are randomly combined in the polydoma itself.

The polydomas can be cultured <u>in vitro</u> or grown <u>in vivo</u> in either genetically compatible animals or nude mice to obtain large quantities of the hybrid antibody which is recovered from the culture medium or ascitic fluid of the animal using known processes. See, for example, the protocols in "Monoclonal Antibodies", Edited

by Kennett et al, Plenum Press, New York (1980) at pp. 363-418.

The mixture of antibodies produced by the polydoma can be resolved to obtain the hybrid. For example, sequential affinity chromatography against first one and then the other antigen for which the hybrid is specific permits its separation from the mono-specific antibody contaminants. We have also found that simple ion exchange chromatography and electrophoretic techniques can be employed as well in at least certain circumstances. If required, the charge difference for ion exchange could b one of the characteristics of the antibody considered in selecting the parental lines.

Example 1

A hybrid monoclonal antibody having a dual specificity for hepatitis B surface antigen (HBsAg) and prostatic acid phosphatase (PAP) was made in accordance with the present invention in the following manner:

A hybridoma secreting a monoclonal antibody to PAP was grown in HAT medium for one week and then transferred to and grown in a non-selective medium. After various lengths of time of growth under non-selective conditions, 2 ml aliquots of cells were placed in medium containing 10^{-4} M 8-azaguanine which prevented cells from growing by incorporating 8-azaguaine in their DNA instead of guanine. Cells lacking the HPRT enzyme survived and grew in this medium and these cells necessarily did not survive in HAT.

Clones that grew in the medium containing 8-azaguanine were tested for sensitivity to HAT and anti-PAP production. One clone which still produced anti-PAP and exhibited HAT sensitivity with a reversion frequency of less than 4×10^{-8} was subcloned. All of the subclones behaved like the parental clone.

Cells from one of the HAT sensitive subclones were fused in polyethylene glycol with spl en cells obtained

from Balb/c mic hyperimmunized with HBsAg to obtain polydomas using the fusion technique of Gerhard. See "Monoclonal Antibodies", supra, at p. 370. The fusion produced 220 polydomas which were screened to determine which secreted antibodies exhibiting specificity for both PAP and HBsAg. Clones of two such polydomas were determined to produce antibody and, subsequently, ascites which exhibited both specificities.

Subclones of both polydomas continued to produce ascites exhibiting both specificities and yielded triple bands on Orstein-Davis PAGE like those of the parent clone. The ascites from both clones were shown to bind ^{125}I-HBsAg and ^{125}I-PAP in radioimmunoassays and yielded Ka values of approximately 10^9 for each, thus suggesting the formation of antibodies having two specificities.

The data in Table I below show the results obtained in immunoassays using the ascites obtained from a clone of one of the polydomas compared with ascites obtained from hybridomas secreting monoclonal antibodies, respectively, against IgE (used as a control), PAP and HBsAg using immobilized HBsAg as a solid phase and a variety of radiolabeled antigens as the solution phase.

A 200 µl sample of the ascites from the polydoma and each of the three hybridomas were each incubated overnight with 12 polystyrene balls to which was bound HBsAg. The HBsAg balls were obtained from Abbott Laboratories, North Chicago, Illinois. After washing, triplicate samples were incubated for 4 hours with 100,000 cpm of the indicated ^{125}I labeled antigen. After a second washing, the balls were counted to determine the amount of labeled antigen bound to the balls. In one set of tests using radiolabeled PAP as the solution phase antigen, anti-PAP was added to the antigen before it was incubated with the ball. The antibody to PAP used for this purpose is the monoclonal antibody produced by the parental hybridoma used to make the polydoma and, therefore, is against the

same PAP epitope as that expected to be exhibited by the hybrid antibody.

TABLE I

Results of Radioassays Demonstrating Presence of Hybrid Antibody Having Dual Specificity in Polydoma Ascites

Specificity of Ascites	cpm *HBsAg	cpm *PAP	cpm *PAP + anti-PAP	cpm *IgE
anti-IgE	15,340	2,145	2,930	3,290
anti-PAP	16,280	2,956	3,128	3,180
anti-HBsAg	73,020	2,973	2,870	3,330
suspected dual specificity	78,900	82,533	2,936	3,143

* ^{125}I labeled antigen

The data in Table I indicate that only the radiation expected from non-specific binding is measured for the anti-PAP ascites when compared to that for the IgE control. The ascites containing the HBsAg antibody, on the other hand, bound the labeled HBsAg antigen as expected but exhibited non-specific binding when the other labeled antigens were tested. The ascites from the polydoma clone, however, bound both labeled HBsAg and labeled PAP, the former attributable to the presence of some non-hybrid, mono-specific antibody to HbsAg in the ascites and the latter attributable to a hybrid that can bind and bridge the HBsAg on the ball and the trace labeled PAP in solution. The experiment using a mixture of labeled PAP and anti-PAP from the parental hybridoma confirms that the anti-PAP specificity of the hybrid is for the same epitope as the antibody secreted by the parent since only background radiation is observed due to inhibition by the parental antibody of binding of labeled PAP to the hybrid antibody.

Analysis of the ascites from the polydoma clone used in the comparative radioassays was performed using polyacrylamide gel electrophoresis (PAGE) and immunoelectrophoresis (IEP). Both indicated the presence of at least three antibody species. Preparative scale DEAE ion exchange chromatography yielded three well separated peaks, the middle one of which had a shoulder. Each of the peaks was homogenous as analyzed by PAGE and IEP, and each corresponded to one of the bands in the original ascites.

Material representing each of the DEAE peaks was tested for antigen binding using radiolabeled HBsAg and PAP. The first peak bound HBsAg but not PAP. The middle peak and its shoulder bound to both HBsAg and PAP and the last peak bound only PAP. Thus, the middle peak is hybrid antibody having a dual specificity to HBsAg and PAP comprised of at least two subspecies.

The hybrid antibody obtained as the middle peak of the DEAE chromatography was radiolabeled with ^{125}I. After labeling, 85% of the labeled antibody would bind to PAP and 88% would bind to HBsAg. The affinity of the hybrid for PAP was found to be slightly lower than that of the monoclonal antibody to PAP produced by the parental line. This difference in affinity was about the same as that observed by us between a monoclonal antibody and its Fab fragment.

DEAE chromatography indicates that hybrid antibody comprises more than 50% of the antibodies produced by the polydoma and roughly approximates the ratio 2:1:1 predicted on statistical grounds if the polydoma were to synthesize all the possible antibody heavy chains, i.e., those exhibiting either PAP or HBsAg specificity, which are combined within the cell on a random basis to form hybrid antibody admixed with lesser amounts of the two mono-specific antibodies having the same specificity as those produced by the parent cells. The existence of

subspecies of hybrid antibody suggests that they may differ in their light chain composition.

Example 2

Hybrid monoclonal antibodies having dual specificity for human IgD and prolactin were made in accordance with the present invention by the fusion of two hybridomas, one of which was constructed to contain two selectible genetic markers: sensitivity to HAT medium and resistance to ouabain. This doubly-marked hybridoma or so-called "universal parent" could then be fused to any other hybridoma. The resulting polydomas grow in the presence of HAT and ouabain, while any unfused parent cells die. The advantages of using such a "universal parent" have been described elsewhere herein.

To construct such a universal parent, both selectible markers were introduced during initial construction of the hybridoma. To obtain this parent cell line, the widely available HAT-sensitive mouse myeloma P3.653 was selected for a second genetic marker, ouabain resistance, by introducing 1 mM ouabain into the growth medium. While most cells died, approximately 1/100,000 cells had by random mutation acquired resistance to the drug and so survived and multiplied to form the new myeloma population which was HAT-sensitive and ouabain resistant.

This HAT-sensitive, ouabain-resistant myeloma was then fused with spleen cells obtained from Balb/c mice hyperimmunized with IgD using the previously cited t chnique of Gerhard. Hybrids were selected in HAT medium (without ouabain) and clones were screened for production of monoclonal antibody directed against IgD. From among the positive clones, one which produced an IgG against IgD was selected for further study. This clone was tested for retention of the trait of ouabain resistance by adding 1 mM ouabain to the growth medium. Approximately one-third of the cells retained this genetic marker. When the culture was growing exponentially in ouabain, the cells

were subcloned. Ouabain-resistant subclones were tested for continued production of the monoclonal anti-IgD antibody. One of the subclones was further backselected by the procedure of Example 1 to obtain a population of cells sensitive to HAT. This subclone was grown for two weeks under non-selective conditions and then placed into medium containing 6-thioguanine. As noted above, the mechanism of action of 6-thioguanine is similar to that for 8-azaguanine. Cells which incorporate 6-thioguanine into their DNA instead of guanine will not grow. Cells lacking HPRT enzyme will not utilize 6-thioguanine from the medium and therefore can grow but are consequently sensitive to HAT. This population of the backselected subclone was then itself subcloned in 6-thioguanine and ouabain-containing medium. Subclones were assayed for continued production of the monoclonal anti-IgD antibody. One clone which showed all the desired characteristics--growth in ouabain and 6-thioguanine as well as production of monoclonal anti-IgD was selected to be a so-called "universal parent." This universal parent could then be fused to any other HAT-resistant, ouabain-sensitive hybridoma to produce a polydoma which would express a hybrid antibody, one specificity of which would be anti-IgD. For this purpose, we initially selected a mouse hybridoma which secretes a monoclonal antibody directed against prolactin. The antiprolactin monoclonal antibody is of the same subclass (IgG $_1$) as the anti-IgD expressed by the parent line and it is easily separated from that antibody on Ornstein-Davis gels. Such a separation is indicative of greatly different charge on the antibodies and so should allow easy isolation of a hybrid antibody by DEAE-Sephadex chromatography.

10^7 cells of the HAT sensitive, ouabain-resistant cell line were fused in polyethylene glycol with 10^7 cells producing anti-prolactin. The fused cells wer first grown for three days in HAT medium, then refed with HAT + ouabain medium for three days and finally placed

again in HAT medium. Greater than 600 clones arose from this fusion: 66 clones were randomly selected for analysis. Of these clones 36 exhibited both anti-IgD and anti-prolactin activity.

These clone supernatants were assayed for the presence of hybrid antibody by the following assay. A polystyrene bead coated with another anti-prolactin monoclonal antibody was incubated 5 hours with 200 µl of a 100 ng/ml prolaction solution. The antibody used binds prolactin at a distinct site from that of the antibody produced by the fused hybridoma cell line. The bead was washed, then incubated overnight with the clone supernatants. The next day, following several washes, ^{125}I labeled IgD was added. Hybrid antibody bound to the bead by one functional arm could bind the radiolabeled IgD with the free anti-IgD functionality whereas neither parental type antibody IgD-IgD or Prolactin-Prolactin could form this bridge between the Prolactin bead and ^{125}I-IgD tracer. Results of a typical assay for clones producing hybrid, bifunctional antibody are presented in Table 2 below:

TABLE 2

Results of immunometric assays demonstrating the presence of hybrid antibody having dual specificity for IgD and prolactin in selected polydoma supernatants and ascites.

Clonate #	cpm ^{125}I-IgD tracer bound
1	15339
2	16337
3	22886
4	23356
5	24434
Anti-IgD	9357
Anti-Prolactin	8721

21 of 36 clones exhibited significant bifunctional activity by this assay. Ascites generated from 2 clones available to date have been shown to react in the bifunctional assay. These ascites contain antibodies which separate into three distinct bands on Ornstein-Davis gels: two bands coincide exactly with antibodies produced by the parent hybridomas (anti-IgD and anti-prolactin). The third band migrates midway between the parental monoclonal antibody bands as expected of the hybrid antibody.

That a hybrid monoclonal antibody against IgD and prolactin exhibits a dose response when the amount of prolactin is varied in the assay used to generate the data of Table 2 is shown by the data of Table 3 using the antibody of clonate #2 and, as controls, antibodies from the parent cell lines (anti-IgD and antiprolactin).

TABLE 3

Results of Assays Using Hybrid Antibody and Varying Amounts of Prolactin

Prolactin ng/ml	Hybrid Antibody[1]	Anti-IgD[1]	Anti Prolactin[1]
0	8010	6847	8020
10	9169	7982	7405
50	13558	7783	8314
100	17599	7654	7844

1. cpm of ^{125}I-IgE bound

These data demonstrate that the amount of prolactin bound by the hybrid antibody is dose responsive as the amount of labeled IgD bound increases as the dose of prolactin is increased. By contrast, no dose response is observed using the parent antibodies against IgD and prolactin as they cannot form the bridge between prolactin

bound to the bead and the labeled IgD. Thus, the hybrid antibody can be used as a component of an assay for prolactin. Tailor-making other hybrid antibodies offers similar opportunities for other assays.

Example 3

By a similar technique to that of Example 2, a universal parent hybridoma was generated which secretes monoclonal antibody directed against the hapten arsenat - arsenate dimer and is both resistant to ouabain and sensitive to HAT. This hybridoma was fused to a hybridoma which secretes a monoclonal antibody with specificity for carcinoembryonic antigen (CEA), an antigen expressed by both embryonic tissues and several types of carcinoma. Once again greater than 600 clones resulted from the fusion. Of 72 clones tested for the ability to bind both CEA and arsenate, 69 had both binding activities. Those clones exhibiting the greatest binding were selected for enzyme-linked immunosorbent (ELISA) assay of hybrid antibody. For each assay, a CEA solution (600 ng or 250 ng) was allowed to adsorb overnight to each well of a plastic 96-well microtiter plate. The next day, unadsorbed material was washed out of the wells with PBS-Tween 20. Clone supernatants were added and incubated 2-1/2 hours at 35°C and then washed off the plate. CEA-CEA and CEA-arsenate antibody would remain attached to the plate via the adsorbed antigen. The second antigen, arsenylic acid coupled to the enzyme alkaline phosphatase, was added to the wells for 3 hours at 35°C. After anoth r wash with PSB-Tween, a chromagen substrate for alkaline phosphatase; para-nitrophenylphosphate, was added to the w lls and color developed for 48 hours. Absorbance in each well was measured at 410 nm. If present in a clone supernatant, hybrid antibody bound the adsorbed CEA on the plate through one functionality, leaving the other free to bind arsenylic acid coupled to alkaline phosphatase. Color from the chromagen substrate developed only wher

th alkaline phosphatase coupled to arsenylic acid had been bound. In this assay 3 of 12 supernatants exhibited hybrid antibody activity as indicated in Table 4.

TABLE 4

Demonstration of hybrid antibody with dual specificity by enzymelinked immunosorbance (ELISA) assay.

Clone	Absorbance at 490 nm 600 ng CEA/well	Absorbance at 490 nm 250 ng CEA/well
1	0.087, 0.105	0.022, 0.060
2	0.082, 0.054	0.023, 0.033
3	0.011, 0.017	0.041, 0.036
anti-arsenate	0.010, 0.006	0.006, 0.005

The present invention also provides methods for immunodiagnosis and immunotherapy using antibodies having a dual specificity, for example, hybrid antibodies obtained as described above or from antibody half molecules by the conventional technique of Nisonoff et al, supra, or antibody multimers obtained by coupling or crosslinking individual monospecific antibodies. Preferably, the antibody having a dual specificity used in these methods is a hybrid antibody prepared according to the present invention as such an antibody can be reliably obtained as a substantially pure compound which has not suffered denaturation and which has a uniform specificity and affinity for the antigen.

In the case of immunodiagnostic applications, one of the two specificities exhibited by the hybrid or other antibody of dual specificity will be against the target antigen whose detection is desired and the other against another antigen, which may be a hapten or other molecular species, which permits the diagnosis. For example, an antibody useful in immunohistology would have a first specificity for a suspect antigen, for example, a tumor associated antigen such as CEA, PAP or ferritin, and a second specificity against a hapten or antigen which

will participate in a color reaction such as an enzyme which causes a color reaction in the presence of a suitable substrate. Among suitable enzymes to which the second specificity of the antibody may be directed is prostatic acid phosphatase (PAP), horse radish peroxidase, glucose oxidase, and alkaline phosphatase.

To perform the histological examination, a tissue section is first treated with the antibody of dual specificity. Prior to doing so, the hybrid can have already been allowed to bind the enzyme which catalyzes the staining reaction. If not, the section is then treated with a second solution containing the enzyme and rinsed after an appropriate incubation and then treated with the substrate which undergoes a color change in the presence of the enzyme. The formation of the color produced by the enzyme and substrate in the tissue sample is a positive indication of the presence in the tissue of the target antigen. The hybrid antibody against HBsAg and PAP whose preparation is described herein, has been found to bind to HBsAg on a test substrate (polystyrene balls) and to PAP in a simulated staining experiment using p-nitrophenyl phosphate as the enzyme substrate. After incubation of the hybrid antibody with PAP and the HBsAg, the addition of the p-nitrophenyl phosphate resulted in the balls undergoing the characteristic yellow to brown color change.

As noted in Example 2, an antibody of dual specificity can also be used in immunoassays and immunometric assays. Using the hybrid antibody against HBsAg and PAP whose preparation is described above, an immunometric assay for HBsAg can be performed using an immobilized monoclonal antibody to HBsAg as a solid phase to extract HBsAg from a serum or other liquid sample suspected of containing the antigen. The sample is incubated with a ball, beads, test tube or other substrate which has the anti-HBsAg bound or coated on its surface. The incubation

with the serum sample can be followed by, done simultaneously with or preceded by an incubation with a solution of the hybrid. In any case, if HBsAg is present in the sample, the result will be the formation of a sandwich of the immobilized antibody, HBsAg if present in the sample, and the hybrid antibody. As part of the assay, PAP is permitted to bind with the hybrid antibody. This can be done during or after formation of the sandwich, or in the alternative, the antibody-PAP complex can be preformed. After formation of the sandwich, the solid phase is washed to remove sample residue and unbound hybrid antibody and then contacted with a solution containing a substrate such as p-nitrophenyl phosphate or α-napthol phosphate which undergoes a color change in the presence of PAP. Occurrence of the color change confirms the presence of target antigen in the sample.

In such an assay, using the polyclonal anti-HBsAg bead of a commercial kit for diagnosis of HBsAg manufactured by Abbott Laboratories of North Chicago, Illinois (sold under the name "Aushia"), samples containing various amounts of HBsAg were incubated with the bead. The samples used were the positive and negative controls from the commercial kit and two samples obtained by diluting the positive control with negative control in the ratios of either 1 part negative control:2 parts positive control or 2 parts negative control:1 part positive control.

After incubation of the samples with the bead to bind HBsAg, the bead was washed and incubated with the hybrid antibody reactive to both HBsAg and PAP. This allowed a sandwich of immobilized antibodies:antigen:and hybrid antibody to form. The bead was washed again and incubated with a solution of PAP. This incubation was followed by another wash and the bead incubated with a substrate α-napthol phosphate. PAP enzymatically removes phosphate. After an appropriate incubation, the substrate was removed from the bead and to it was added an indicator Fast Garnet GBC salt which turns from clear to a reddish

purple in the presence of the product of the enzymatic reaction. A color change was observed to confirm the presence of HBsAg in the samples. Absorbance at 570 nm was measured for the samples and these data are shown in Table 5 below.

TABLE 5

HBsAg Concentration (% of Positive Control)	Absorbance 570nm
0	.031
34	.166
67	.243
100	.379

These data show a dose response with variation in HBsAg concentration which would be expected if the hybrid antibody forms a bridge between HBsAg bound to the ball and PAP. This further demonstrates the utility of a hybrid antibody in an immunoassay.

Detection means other than enzymatically catalyzed reactions are also possible. For example, the second specificity of the hybrid or other antibody having a dual specificity can be directed against a hapten or antigen which is radiolabeled or which is fluorescent or which is detectable in the sandwich by any other suitable means.

A preferred process which utilizes a hybrid antibody or other antibody having a dual specificity in an immunoassay exploits the phenomenon of fluorescence quenching. In such an assay, one specificity of the antibody is directed against a target antigen and the other against, for example, a hapten bearing a fluorescing chromophore. The chromophore is either bound to the hapten or, in appropriate cases, may be the hapten itself.

The assay is conducted by incubating the antibody with serum or other sample suspected of containing the target antigen to which has been added a predetermined quantity of target antigen labeled with a quenching

chromophore. The labeled antigen competes with target antigen in the sample, if any, for the antibody binding site specific for the target antigen. Before, during or after this incubation, a quantity of the hapten bearing the fluorescing chromophore is incubated with the antibody and binds at the other binding site.

The two chromophores are selected so that the first of them fluoresces at a wavelength which can be absorbed (quenched) by the other if they are positioned closely enough together so that the photon emitted by the fluorescer can be captured by the quencher. To do this, the two chromophores should be within about 100 angstroms and, preferably, within about 50 angstroms of each other. This positioning will occur when the fluorescing chromophore is bound at one antibody binding site and the quenching chromophore is bound to added antigen at the other. A suitable pair of chromophores includes fluorescein as the fluorescing chromophore and rhodamine as the quenching chromophore.

The measured fluorescence will vary inversely with the amount of native antigen in the sample since, in the absence of native antigen, all of the antigen bound to the antibody will be labeled with the quenching chromophore and be positioned to absorb fluorescence by the chromophore carried by the hapten. Comparison of the measured fluorescence with that of a control sample containing a known amount of antigen permits a qualitative and quantitative determination of the presence of antigen in the sample.

This kind of immunoassay can, for example, be used to determine the levels in serum of drugs such as dilantin which must be closely monitored. In such an assay, the target antigen would, of course, be dilantin. It will be apparent to those skilled in the art that this process can be used to detect other antigens as well including, in particular, tumor associated antigens.

Another preferred process which utilizes a hybrid antibody or other antibody having a dual sp cificity in an immunoassay relies upon an enzymatic reaction. In a presently preferred process, one of the antibody specificities is directed, of course, to the target antigen and the other to an enzyme or a hapten to which is bound an enzyme.

The assay is conducted by incubating the antibody with a sample suspected of containing the target antigen to which has been added a predetermined quantity of th target antigen that has been modified by binding to it a substance that interacts with the enzyme to produce either a detectable substance or in some other way to permit detection of formation of the antigen-antibody complex. Detection may be, for example, by fluorimetry, luminescence, spectrophotometry or the like.

In an alternative process, the added target antigen may have the enzyme bound to it in which case the antibody has one of its specificities directed against the substance which interacts with the enzyme or against a hapten to which the substance is bound.

The substance which interacts with the enzyme can itself be another enzyme. In such a case, one of the enzymes catalyzes production of a product required by the other. Thus, when the antibody binds both the added target antigen, to which is bound one of the enzymes, and the other enzyme, the product of the first enzymatic reaction is formed in proximity to the second enzyme and can undergo a reaction catalyzed by the latter enzyme before significant diffusion of the product into the surrounding medium can occur.

An example of such a process utilizes the two enzymes hexokinase (HK) and glucose-6-phosphate dehydrogenase (G-6-PDH) in the following reaction scheme.

HK

(1) adenosine triphosphate + glucose ——————

(ATP)

adenosine diphosphate + glucose-6-phosphate

(ADP)

(2) glucose-6-phosphate + nicotinamide adenine dinucleotide

(NAD+)

6-6-PDH

——————— gluconolactone-6-phosphate + dihydronicotinamide adenine dinucleotide

(NADH)

To exploit this reaction scheme, the added target antigen will have either HK or G-6-PDH bound to it and the hybrid antibody will have one of its specificities directed against the other (or a hapten bearing it). The sample has added to it, in addition to the hybrid antibody and the predetermined amount of enzyme labeled antigen, glucose, ATP and the coenzyme NAD^+. The hybrid antibody preferably has it the other enzyme already bound to it. Alternatively, this enzyme can be added to the sample with the other reagents.

During the incubation, the enzyme labeled antigen will compete with native antigen in the sample, if any, for one of the hybrid antibody binding sites. The other enzyme is or will be, bound to the second binding site. This permits the formation of glucose-6-phosphate catalyzed by HK to occur in close proximity to G-6-PDH. The latter converts the glucose-6-phosphate to gluconolactone-6-phosphate, a result which is accompanied by the reduction of NAD^+ to NADH. The NADH absorbs strongly at 340 nm and, therefore, can be detected spectrophotmetrically. The amount of NADH formed varies inversely with the amount of native antigen in the sample, i.e., its maximum production occurs when there is no target antigen in the sample being assayed. Comparison of the amount of the NADH formed with a control sample permits a qualitative

and quantitative determination of the presenc of antigen in the sample.

This kind of assay can be used to monitor the level of dilantin or other drugs in serum. In such a case, the drug is the target antigen. However, such an assay can also be used to detect other serum antigens such as those associated with tumors or other diseases.

In vivo immunodiagnosis can also be performed using a hybrid or other dual specificity antibody. The antibody, having one specificity against a target antigen such as a tumor associated antigen and the second against a hapten to which is bound a suitable radionuclide, preferably one which emits γ-radiation, is first administered to the host. After a sufficient time has passed during which the antibody has localized at the target site and unbound antibody has been permitted to clear from healthy tissue in the host, the hapten bearing the radionuclide is administered and binds to the localized antibody. After a suitable interval to permit unbound hapten to clear the host, scanning of the host with a suitable camera is conducted to determine whether there are areas in which radiation has been concentrated. If there are any, the presence of the target antigen in the host is confirmed and its position determined.

This process has several advantages over that using monospecific antibody directed against the target antigen to which the radionuclide is directly bound. In such cases, the radionuclide must have a long enough half life that a sufficient quantity remains after the time necessary for substantial localization of the antibody at the target site has elapsed. Furthermore, during this process the antibody may be retained for a period of time in the liver or other non-target tissues which are then subjected to the radiation carried by the antibody. The present invention, on the other hand, permits the use of radionuclides having shorter half-lives than those used with monospecific antibodies. Being a relatively small

particle, the radionuclide bearing hapten has a high mobility in vivo and will travel rapidly through the host and either bind to the antibody which has localized at the target site or clear the body without spending appreciable time in non-target tissue. For this reason, isotopes of short half-life can be administered in quantities which pose the minimum risk to healthy tissue even though administered in substantial excess.

Preferably, the hapten is an agent to which the radionuclide is directly bound or which will complex with the radionuclide. A chelating agent for the radionuclide bound to a hapten may be used for the latter purpose. Those skilled in the art will appreciate that a wide variety of chelating agents and radionuclides are suited for this purpose. Phenylarsenate to which ethylenediaminetetraacetic acid (EDTA) is bound as a chelating agent is a suitable hapten. A radionuclide suited for use with this hapten is ^{111}In.

The antibody of dual specificity can also be used in immunotherapy by constructing it to have one specificity against a disease associated antigen and the other against a hapten which is, or to which is bound, an agent lethal to the antigen or diseased tissue with which the antigen is associated and which it is desired to destroy. For example, the antibody may have one specificity against a tumor associated antigen such as PAP, carcinoembryonic antigen (CEA), ferritin, or other such antigen and a second specificity directed to a hapten to which is bound a radionuclide, preferably one which emits or -radiation, or is comprised of a ricin A chain or other toxin or drug. Among such drugs may be mentioned gelonin, α-amanitin, diphtheria toxin A, methotrexate, dichloromethatrexate, dounomycin and chlorombucil. Of course, if the toxin or drug can itself function as a hapten, it need not be bound to any other moiety.

In the case where a radionuclide is to be used as the lethal agent, just as in the situation wher they are used for in vivo immunodiagnosis, the hapten can have the radionuclide bound directly to it or the hapten can be, or have bound to it, an agent such as a chelating agent which will form a complex with the radionuclide. In such a case, the hybrid or other antibody of dual specificity is administered to the diseased host and allowed to localize at the site of the affected tissue and any excess allowed to clear the host, followed by administration of the hapten which is bound by the antibody wherever it has localized. This permits the use of a radionuclide having a short half-life which minimizes the risk of injury to healthy tissue even though the radionuclide bearing hapten is administered in substantial excess since that excess will rapidly clear the body and not localize in substantial quantities in healthy tissue because of the hapten's relatively small size. It also eliminates or reduces the possibility that circulating target antigen will bind antibody bearing a substance lethal to tissue and deliver it to healthy tissue as can occur when the lethal agent is bound directly to a monospecific antibody directed against the target antigen.

An example of a hapten to which a radionuclide is directly bound is 6-^{211}At-astato-2-methyl-1,4-naptho-quinol bis(disodium phosphate) which is described in "International Journal of Applied Radiation and Isotopes", 33, 75 (1982). The ^{211}At is an emitter of α-radiation. Those skilled in the art will appreciate that there are numerous suitable radionuclides which can be bound directly to haptens or complexed with a hapten by means of any of a wide variety of chelating agents.

The foregoing description of the invention is of presently preferred embodiments. Variations are possible without departure from the scope of the invention which is limited only by the appended claims.

Claims:

1. A polydoma which produces a hybrid monoclonal antibody having a dual specificity.

2. A polydoma according to Claim 1 which produces the hybrid antibody as a component of a mixture of antibodies.

3. A polydoma according to Claim 2 wherein the mixture of antibodies comprises the hybrid antibody and two species of mono-specific antibody.

4. A polydoma according to Claim 3 wherein the hybrid antibody is comprised of two subspecies.

5. A polydoma according to Claim 4 or 5 wherein the specificity of one of the mono-specific antibodies is the same as one of the dual specificities of the hybrid and the specificity of the other mono-specific antibody is the same as the other specificity of the hybrid antibody.

6. A hybrid monoclonal antibody having a dual specificity produced by a polydoma.

7. A hybrid monoclonal antibody according to Claim 6 wherein one of the dual specificities is against a target antigen and the other against a substance which permits diagnosis of the target antigen.

8. A hybrid monoclonal antibody according to Claim 7 wherein the substance permitting diagnosis is radiolabelled.

9. A hybrid antibody according to Claim 8 wherein the substance permitting diagnosis is a hapten.

10. A hybrid antibody according to Claim 9 wherein the radiolabel is a radionuclide bound directly to the hapten.

11. A hybrid antibody according to Claim 9 wherein the radionuclide is bound to th hapten by a chelating agent.

12. A hybrid monoclonal antibody according to Claims 8, 9, 10 and 11 wherein the radiolabel is an emitter of γ-radiation.

13. A hybrid monoclonal antibody according to Claim 8 wherein the substance is fluorescent.

14. A hybrid monoclonal antibody according to Claim 9 wherein the hapten is fluorescent.

15. A hybrid monoclonal antibody according to Claim 9 wherein a fluorescent moiety is bound to the hapten.

16. A hybrid monoclonal antibody according to Claim 8 wherein the substance permitting diagnosis is an enzyme.

17. A hybrid monoclonal antibody according to Claim 9 wherein an enzyme is bound to the hapten.

18. A hybrid monoclonal antibody according to Claims 7, 8, 9, 10, 11, 13, 14, 15, 16 or 17 wherein the target antigen is a disease associated antigen.

19. A hybrid monoclonal antibody according to Claim 18 wherein the antigen is a tumor associated antigen.

20. A hybrid monoclonal antibody according to Claim 6 wherein one of the dual specificities of the antibody is against a target antigen and the other against a hapten which is, or to which is bound, an agent lethal to the antigen or associated tissue.

21. A hybrid monoclonal antibody according to Claim 20 wherein the lethal agent is a radionuclide.

22. A hybrid monoclonal antibody according to Claim 21 wherein the radionuclide is bound directly to the hapten.

23. A hybrid monoclonal antibody according to Claim 21 wherein the radionuclide is bound to the hapten by a chelating agent.

24. A hybrid monoclonal antibody according to Claims 21, 22, or 23 wherein the radionuclide is an emitter of α-radiation or β-radiation.

25. A hybrid monoclonal antibody according to Claim 20 wherein the lethal agent is a tissue toxin.

26. A hybrid monoclonal antibody according to Claim 25 wherein the tissue toxin is comprised of a ricin A chain.

27. A hybrid monoclonal antibody according to Claims 20, 21, 22, 23, 25 or 26 wherein the target antigen is a disease associated antigen.

28. A hybrid monoclonal antibody according to Claim 27 wherein the antigen is a tumor associated antigen.

29. A process for producing a polydoma which produces a hybrid monoclonal antibody having a dual specificity which comprises fusing a hybridoma which produces a monoclonal antibody against a first antigenic determinant with a B-lymphocyte which secretes a monoclonal antibody against a second antigenic determinant in the pres nce of a fusion promoting agent.

30. A process according to Claim 29 wherein the hybridoma is selectively destructible.

31. A process according to Claim 30 wherein the hybridoma has been back selected to obtain cells that are sensitive to a medium in which the polydoma can be cultured.

32. A process according to Claim 31 wherein back selection is accomplished by culturing cells of the hybridoma in a medium comprising a member of the group consisting of 8-azaguanine, 6-thioguanine, 5-bromouracyl deoxyribose or 2-aminopurine whereby hybridoma cells sensitive to medium containing hypoxanthine aminopterin thymidine are obtained.

33. A process according to Claim 30 wherein said selectively destructible hybridoma is obtained by irreversible enzyme inhibition.

34. A process according to Claim 33 wherein the inhibition is obtained using a metabolic inhibitor.

35. A process according to Claim 34 wherein the inhibitor is a K_{cat} inhibitor.

36. A process according to Claim 35 wherein the K_{cat} inhibitor is selected from azaserine or 5-diazo-5-oxa-L-norleucine.

37. A process according to Claims 29, 30, 31, 32, 33, 34, 35 or 36 wherein the B-lymphocyte is a mammalian spleen cell.

38. A process according to Claim 37 wherein the spleen cell is a spleen cell of a murine specie.

39. A process for producing a polydoma which secretes a hybrid monoclonal antibody having a dual specificity which comprises fusing a first hybridoma which secretes a

monoclonal antibody against a first antigenic determinant with a second hybridoma which secretes a monoclonal antibody against a second antigenic determinant in the presence of a fusion promoter.

40. A process according to Claim 39 wherein the first and second hybridomas are selectively destructible.

41. A process according to Claim 40 wherein at least one of the hybridomas has been back selected to obtain cells that are sensitive to a medium in which the polydoma can be cultured.

42. A process according to Claim 41 wherein both of the hybridomas have been back selected.

43. A process according to Claim 40 wherein back selection is accomplished by culturing cells of the hybridoma in a medium comprising 8-azaguanine, 6-thiguanine, 5-bromouracyl or 2-aminopurine whereby hybridoma cells sensitive to medium containing hopyxanthine aminopterin thymidine are obtained.

44. A process according to Claim 39 wherein said selectively destructible hybridomas are obtained by irreversible enzyme inhibition.

45. A process according to Claim 44 wherein the inhibition is obtained using a metabolic inhibitor.

46. A process according to Claim 45 wherein the inhibitor is a K_{cat} inhibitor.

47. A process according to Claim 46 wherein the inhibitor is selected from azaserine or 5-diazo-5-oxa-L-norleucine.

48. A process according to Claim 39 wherein only the first hybridoma is selectively destructible.

49. A process according to Claim 48 wherein the selectively destructible hybridoma is further selected to confer the ability for it to survive in a medium which is lethal to the other hybridoma.

50. A process according to Claim 49 wherein the selectively destructible hybridoma is resistant to medium containing ouabain.

51. A process according to Claim 50 wherein the hybridoma is back selected to obtain cells that are sensitive to a medium in which the polydoma can be cultured.

52. A process according to Claim 51 wherein back selection is accomplished by culturing cells of the hybridoma in a medium comprising a member selected from the group consisting of 8-azaguanine, 6-thioguanine, 5-bromouracyl deoxyribose or 2-aminopurine whereby hybridoma cells sensitive to medium containing hypoxanthine aminopterin thymidine are obtained.

53. A process according to Claim 50 wherein the selectively destructible hybridoma is obtained by irreversible enzyme inhibition.

54. A process according to Claim 53 wherein the inhibition is obtained using a metabolic inhibitor.

55. A process according to Claim 54 wherein the inhibitor is a K_{cat} inhibitor.

56. A process for producing a polydoma which secretes a hybrid monoclonal antibody having a dual specificity

which comprises removing the nucleus from a first hybridoma cell which produces a monoclonal antibody against a first antigenic determinant and inserting the nucleus into the cytoplasm of a second hybridoma which produces a monoclonal antibody against a second antigenic determinant.

57. A process for producing a hybrid monoclonal antibody having a dual specificity comprising isolating the antibody from cells of a polydoma produced according to the process of Claims 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55 or 56.

58. An immunometric process comprising:

a) administering to a host an antibody having a dual specificity, one specificity of which is directed against a disease associated antigen and the other against a hapten, the hapten being an agent lethal to the antigen or associated tissue or having a lethal agent bound to it; and

b) administering the hapten after sufficient time has elapsed to permit the antibody to bind to the disease associated antigen.

59. A process according to Claim 58 wherein the lethal agent is a radionuclide.

60. A process according to Claim 59 wherein the radionuclide is an emitter of α-radiation or β-radiation.

61. A process according to Claim 58 wherein the lethal agent is a tissue toxin.

62. A process according to Claim 61 wherein th toxin is comprised of a ricin A chain.

63. A proc ss according to Claims 58, 59, 60, 61 or 62 wherein the antigen is a tumor associated antigen.

64. A process according to Claims 58, 59, 60, 61 or 62 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

65. A process according to Claim 63 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

66. A process according to Claims 58, 59, 60, 61 or 62 wherein the antibody is a hybrid antibody produced by the reassociation of antibody half molecules obtained by the selective cleavage of a mono-specific antibody against the disease associated antigen and a mono-specific antibody against the hapten.

67. A process according to Claim 66 wherein the selectively cleaved antibodies are monoclonal antibodies.

68. A process according to Claim 66 wherein the selectively cleaved antibodies are polyclonal antibodies.

69. A process according to Claims 58, 59, 60, 61 or 62 wherein the antibody is a multimer of a pair of intact mono-specific antibodies, one of said mono-specific antibodies being against the disease associated antigen and the other against the hapten.

70. A process according to Claim 69 wherein the mono-specific antibodies are monoclonal antibodies.

71. A process according to Claim 69 wherein the mono-specific antibodies are polyclonal antibodies.

72. An in vivo immunodiagnostic process comprising:

a) administering to a host an antibody having a dual specificity, one specificity of which is directed against a disease associated antigen and the other against a hapten bearing a radionuclide;

b) administering the hapten after sufficient time has elapsed to permit the antibody to bind to the disease associated antigen; and

c) scanning the host to detect the location of radiation emitted by the radionuclide.

73. A process according to Claim 72 wherein the radionuclide is an emitter of γ-radiation.

74. A process according to Claim 72 wherein the radionuclide is bound directly to the hapten.

75. A process according to Claim 72 wherein the radionuclide is bound to the hapten by a chelating agent.

76. A process according to Claims 72, 73, 74 or 75 wherein the antigen is a tumor associated antigen.

77. A process according to Claims 72, 73, 74 or 75 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

78. A process according to Claim 76 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

79. A process according to Claims 72, 73, 74 or 75 wherein the antibody is a hybrid antibody produced by the reassociation of antibody half molecules obtained by the selective cleavage of a mono-specific antibody against the disease associated antigen and a mono-specific antibody against the hapten.

80. A process according to Claim 79 wherein the selectively cleaved antibodies are monoclonal antibodies.

81. A process according to Claim 79 wherein the selectively cleaved antibodies are polyclonal antibodies.

82. A process according to Claims 72, 73, 74 or 75 wherein the antibody is a multimer of a pair of intact mono-specific antibodies, one of said mono-specific antibodies being against the disease associated antigen and the other against the hapten.

83. A process according to Claim 82 wherein the mono-specific antibodies are monoclonal antibodies.

84. A process according to Claim 82 wherein the mono-specific antibodies are polyclonal antibodies.

85. An immunoassay process comprising:

a) adding to a sample suspected of containing a target antigen a predetermined amount of the target antigen to which is fixed a chromophore;

b) adding to the sample an antibody having a dual specificity, one specificity of which is directed against the target antigen and the other against a hapten which is, or to which is bound, a fluorescing chromophore which chromophore fluoresces at a wavelength that is absorbable by the chromophore on the target antigen when said chromophores are within about 100 A of each other;

c) binding the hapten to the antibody;

d) measuring the fluorescence of the sample after a period of incubation;

e) comparing the intensity of fluorescence of the sample with that of a control sample containing a known amount of target antigen.

86. A process according to Claim 85 wherein th antigen is a tumor associated antigen.

87. A process according to Claim 85 wherein the hapten is bound to the antibody before the antibody is added to the sample.

88. A process according to Claim 86 wherein the hapten is bound to the antibody before the antibody is added to the sample.

89. A process according to Claims 85, 86, 87 or 88 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

90. A process according to Claims 85, 86, 87 or 88 wherein the antibody is a hybrid antibody produced by the reassociation of antibody half molecules obtained by the selective cleavage of a mono-specific antibody against the disease associated antigen and a mono-specific antibody against the hapten.

91. A process according to Claim 90 wherein the selectively cleaved antibodies are monoclonal antibodies.

92. A process according to Claim 90 wherein the selectively cleaved antibodies are polyclonal antibodies.

93. A process according to Claims 85, 86, 87 or 88 wherein the antibody is a multimer of a pair of intact mono-specific antibodies, one of said mono-specific antibodies being against the target antigen and the other against the hapten.

94. A process according to Claim 93 wherein the mono-specific antibodies are monoclonal antibodies.

95. A process according to Claims 85, 86, 87 or 88 wherein the fluorescing chromophore is fluorescein and the quenching chromophore is rhodamine.

96. An immunoassay process comprising:

a) adding to a sample suspected of containing a target antigen a predetermined amount of the target antigen to which is bound a substance capable of interacting with an enzyme to produce a detectable product;

b) adding to the sample an antibody having a dual specificity, one specificity of which is directed against the target antigen and the other against the enzyme or a hapten to which the enzyme is bound;

c) binding the hapten to the antibody;

d) measuring the formation of the detectable substance after a period of incubation;

e) comparing the formation of the detectable substance with that of a control sample containing a known amount of target antigen.

97. A process according to Claim 86 wherein the enzyme is bound to the antibody before the antibody is added to the sample.

98. A process according to Claim 91 wherein the antigen is a tumor associated antigen.

99. A process according to Claim 97 wherein the antigen is a tumor associated antigen.

100. A process according to Claims 96, 97, 98 or 99 wherein the antibody is a hybrid monoclonal antibody produced by a polydoma.

101. A process according to Claims 96, 97, 98 or 99 wherein th antibody is a hybrid antibody produced by the reassociation of antibody half molecul s obtained by

the selective cleavage of a mono-specific antibody against the target associated antigen and a mono-specific antibody against the hapten.

102. A process according to Claim 101 wherein the selectively cleaved antibodies are monoclonal antibodies.

103. A process according to Claim 101 wherein the selectively cleaved antibodies are polyclonal antibodies.

104. A process according to Claims 96, 97, 98 or 99 wherein the antibody is a multimer of a pair of intact mono-specific antibodies, one of said mono-specific antibodies being against the disease associated antigen and the other against the hapten.

105. A process according to Claim 104 wherein the mono-specific antibodies are monoclonal antibodies.

106. A process according to Claims 96, 97, 98 or 99 wherein the substance bound to the target antigen is a second antigen and wherein one enzyme catalyzes the formation of a product which interacts with the other enzyme to produce a detectable substance.

107. A process according to Claim 106 wherein the detectable substance is detected by its fluorescence, luminescence or spectroscopically.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 83/00525

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

US: 435/7
IPC:3 G01N 33/54

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
	435/7,68,172,240,241,948 436/548 424/1,1.5,9, 85

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Lexpat 1975-7/1983

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X,A	US,A, 4130634 19 DECEMBER 1978, MOLINARO ET AL	6-28, 58-107
P,X,A	US,A, 4331647, 25MAY 1982, GOLDENBERG	6-28,58-107
A,P	US,A, 4376110, 8 MARCH 1983, DAVID ET AL	58-107
X,A	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, 78(9) SEPTEMBER 1981, KRANZ ET AL, "RESTRICTED REASSOCIATION OF HEAVY AND LIGHT CHAINS FROM HAPTEN-SPECIFIC MONOCLONAL ANTIBODIES", P. 5807-11	1-57
X,A	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES 71(6) JUNE 1974, SCHWABER ET AL "PATTERN OF IMMUNOGLOBULIN SYNTHESIS AND ASSEMBLY IN A HUMAN-MOUSE SOMATIC CELL HYBRID CLONE" p. 2203-7	1-57
X,A	N, HERZENBERG ET AL, "CELL HYBRIDS OF MYELOMAS WITH ANTIBODY FORMING CELLS AND T-LYMPHOMAS WITH T CELLS" IN "HANDBOOK OF EXPERIMENTAL IMMUNOLOGY" EDITED BY WEIR 1979 , BLACKWELL PUBLICATIONS, LONDON, p. 25.1-25.7 NOTE PAGE 25.5 IN PARTICILAR	

* Special categories of cited documents: [15]

"A" document defining the general state of the art
"E" earlier document but published on or after the international filing date
"L" document cited for special reason other than those referred to in the other categories
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but on or after the priority date claimed
"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention
"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21 JULY 1983	27 JUL 1983
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	John E. Tarcza JOHN EDWARD TARCZA

Form PCT/ISA/210 (second sheet) (October 1977)

FURTHER INFORMATI N CONTINUED FR M THE SECOND SHEET

X,A	N, FEDERATION PROCEEDINGS 37, 1978 p. 1350 ABSTRACT #444 "HYBRID ANTIBODIES AS IMMUNO-PHARMACOLOGIC AGENTS", RASO ET AL,	6-28,58-107
A,X	N, CANCER RESEARCH 41, JUNE 1981 RASO ET AL, "HYBRID ANTIBODIES WITH DUAL SPECIFICITY FOR THE DELIVERY OF RICIN TO IMMUNOGLOBULIN BEARING CELLS" p. 2073-8	6-28, 58-107
X,A	N, ROSE ET AL, PRINCIPLES OF IMMUNOLOGY, 2ND EDITION MACMILLIAN PUBLISHING CO. NY, 1979 p. 499-507	6-28,58-107
X,E	EP,A, 0 068763, 1 MAY 1983, READING	1-107

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

I. CLAIMS 1-5 AND 29-57
II. CLAIMS 6-28 AND 58-107

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.